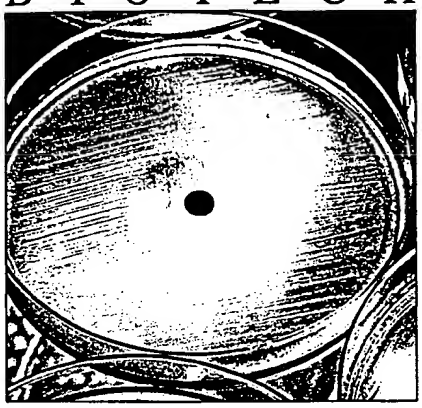
A New Technology for Preparing Monoclonal Antibodies to Molecules Expressed on the Cell Surface

BIOTECH



TRENDS

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Surface-epitope masking (SEM) is a new procedure that permits the efficient and selective production of monoclonal antibodies (MAbs) reactive with both known and unknown molecules expressed on the cell surface. Applications of this technology include the development of MAbs reacting with the multidrug resistance gene, human interferon receptors, novel human prostate carcinoma tumor-associated antigens (TAAs), and human breast carcinoma TAAs. In theory, the SEM approach can be used to develop MAbs and to identify genes associated with important cellular processes including growth, differentiation, immunological recognition, tumorigenesis, metastasis, cell senescence, atypical MDR, and autoimmune diseases.

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The production of monoclonal antibodies (MAbs) targeted toward defined cell surface–expressed molecules is often an inefficient, time-consuming, and expensive procedure (1–3). Recently, a method based on immunological subtraction was devised to circumvent these problems. This approach is called surface-epitope masking (SEM) (4).

The SEM process involves the generation of polyclonal antibodies reacting with a complete and random set of surface antigens expressed on one cell type (referred to as a *driver*). These polyclonal antibodies coat (mask) common surface antigens on a related cell type expressing a surface molecule to be targeted for the production of MAbs (referred to as a *tester*). The SEM procedure allows efficient generation of hybridomas that produce MAbs reacting with cell surface–expressed molecules of both known and unknown functions (4).

A major problem that often limits the effectiveness of chemotherapy is the acquisition by tumor cells of cross-resistance to structurally diverse chemotherapeutic agents, a process called *multidrug resistance* (MDR) (5,6). The genetic lesion that induces one form of MDR — namely, typical MDR — involves increased expression of a gene (mdr-1) that encodes a 170,000 molecular weight cell surface–expressed drug-efflux transporter protein (P-glycoprotein) (5).

Studies have determined whether the SEM strategy could develop MAbs that react specifically with external surface-localized epitopes of the human MDR P-glycoprotein (4). In this process, the driver cell is a cloned rat embryo fibroblast cell line, CREF-Trans 6 (6,7), and the tester cell line is a genetically altered MDR-CREF-Trans 6 cell clone. MDR-CREF-Trans 6 cells are incubated with high-titer polyclonal antibodies produced against the untransfected CREF-Trans 6 parental cells. Surface-epitope-masked MDR-CREF-Trans 6 cells are injected into BALB/c mice, and immune spleen cells taken from these mice are fused with myeloma cells. This process results in the development of hybridomas that produce MAbs that react by fluorescence activated cell sorter (FACS) analysis with tester MDR-CREF-Trans 6 cells, but not with driver non-MDR-CREF-Trans 6 parental cells (4). The ability of these genetically altered MDR-CREF-Trans 6 cells to react with the SEM-derived MDR MAbs by FACS indicates that these antibodies can interact with specific surface-expressed native epitopes on the MDR P-glycoprotein.

To determine the utility and reactivity of the SEM-derived MDR MAbs toward additional cell types expressing the human typical MDR gene, the human breast carcinoma cell line MCF-7 was genetically modified with the human mdr-1 gene (4). Whereas the non-MDR parental MCF-7 cells do not react with the SEM-derived MDR MAbs, all of the independently derived MDR-MCF-7 clones are reactive by FACS analysis with the SEM-derived MAbs. These findings indicate that the SEM approach can target MAb production toward epitopes on a defined human surface-expressed protein.

A further application of the SEM approach for developing MAbs that react with known surface-expressed proteins involves human interferon receptors. In a collaborative project with Dr. Sidney Pestka's laboratory (Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey), CREF-Trans 6 cells were genetically engineered to express either human IFN-γ or IFN-α receptors. CREF-Trans 6 cells expressing the IFN-γ receptors were used as tester cells for application of the SEM procedure. Hybridomas were produced that secrete MAbs reacting with human IFN-γ surface receptors in genetically engineered CREF-Trans 6 cells, human cells, and somatic cell hybrids containing the appropriate human genes and encoding the human IFN-γ receptor. A similar SEM approach is currently being used to develop MAbs that react with human IFN-α receptors and surface-expressed human interferon accessory proteins. These findings provide further scientific support for SEM and for the use of this approach to tailor the development of MAbs that react with specific surface-expressed molecules.

The SEM approach also has wide applicability to the development of MAbs that react with surface-expressed molecules with unknown functions. For example, with human prostate carcinoma DNA transfected nude mouse tumor-derived CREF-Trans 6 cells, the

SEM procedure produced MAbs that react with surface molecules expressed on transfected (genetically altered) but not on untransfected CREF-Trans 6 cells (4). These MAbs, known as Pro 1.1 to Pro 1.5, also react with the surface of human prostate carcinoma cell lines, including LNCaP (initial source of transforming DNA), DU-145, and PC-3 (4). In addition, Pro 1.5 reacts with surface epitopes on human breast carcinoma cells, including MCF-7 and T47D, but not with human skin fibroblasts, specific human colon carcinomas, or melanomas (4). In recent studies, Pro 1.5 has displayed reactivity with fresh-frozen sections of patient-derived prostate carcinomas, but not with normal prostate or benign prostatic hypertrophy tissue.

A similar approach was used to develop MAbs that react with the surface of human breast carcinomas. High molecular weight DNA from the human breast carcinoma cell line T47D was cotransfected with an antibiotic resistance gene (pSV2neo) into CREF-Trans 6, and G418-resistant colonies were injected into athymic mice. Tumors that developed were removed, established in culture, and subjected to the SEM protocol. The SEM-derived MAbs, Br-car 4.2.1 and Br-car 5.2.4, displayed reactivity with the surface of appropriately transfected CREF-Trans 6 cells, as well as T47D and MCF-7 human breast carcinoma cells. Br-car 4.2.1 and Br-car 5.2.4 selectively detected breast carcinomas in both fresh-frozen and formalin-fixed patient-derived specimens. These studies indicate the power of the SEM approach in developing immunological reagents with direct diagnostic potential.

The SEM-derived MAbs can also be used to clone genes that encode unidentified surface-localized molecules expressed by genetically modified CREF-Trans 6 cells. For example, screening of an LNCaP cDNA expression library with Pro 1.5 resulted in cloning of prostate carcinoma tumor-antigen gene-1 (PCTA-1) (unpublished data). PCTA-1 is a novel gene, not reported in current DNA databases, of 3.8 Kb that encodes a protein with a predicted M_r of ~36 Kd. A protein of similar size is detected after in vitro translation of the PCTA-1 cDNA in a rabbit reticulocyte translation system and by immunoprecipitation from ^{35}S-methionine-labeled lysates from LNCaP and DU-145 cells. With specific sequences present in PCTA-1, primers are now available that detect PCTA-1 expression by RT-PCR in transfected cells, prostate carcinoma cell lines, and patient-derived prostate carcinomas. These results indicate that SEM can develop MAbs of clinical interest and can identify genes that encode these proteins.

The SEM protocol may be useful for enhancing the sensitivity of the "phage display combinatorial library" approach to identifying genes involved in transformation and genes that encode surface-expressed human tumor-associated antigens (8,9). For example, human tumor DNA-transfected CREF-Trans 6 cells can be coated with high-titer CREF-Trans 6 polyclonal antibody and used as immunogens to stimulate an immune response in mice. Isolated spleen cells can then directly generate combinatorial phage cDNA libraries. In this way, genes that encode surface-expressed molecules involved in transformation and cell surface–expressed, tumor-associated antigens can be directly identified and cloned.

POTENTIAL APPLICATIONS

Conceptually, SEM represents a broad-based "immunological subtraction" technology. It will permit the directed development of MAbs and the identification of genes reacting with

and encoding, respectively, diverse cell surface–expressed molecules present in a tester target cell but missing in a driver target cell. Although our current use of SEM has involved the CREF-Trans 6 system, the applications of this approach are much broader. Numerous additional applications of the SEM approach are possible. Following are some examples of these applications.

Developing MAbs specific for early-stage and metastatic cancers. Polyclonal antibodies are produced against an early-stage or metastatic tumor of the same histology, followed by the SEM approach to develop, respectively, early-stage- or metastatic-specific MAbs. These SEM-derived MAbs would be useful for diagnostics or, if they displayed appropriate specificity, for therapeutic applications.

Identifying genes encoding surface-expressed molecules found preferentially in early-stage and metastatic cancers. The SEM approach can be coupled with the combinatorial phage cDNA expression library approach to identify cDNAs that encode surface-expressed early-stage cancer or metastatic cancer antigens. The cDNAs can be sequenced and this information can be used to develop primers for RT-PCR, thereby resulting in potentially important cancer diagnostic tools.

Developing MAbs reacting with patient-specific, tumor-associated, cell surface-expressed molecules. To achieve this goal, polyclonal antibodies produced against adjacent normal tissue (e.g., normal breast tissue) mask antigens on genetically matched tumor tissue (e.g., breast carcinoma). SEM-derived MAbs reacting with specific tumor-associated antigens on a patient's carcinomas (which may also represent generic antigens expressed on additional patient breast carcinomas) may prove useful for identifying metastatic spread and for therapeutic applications (immunoconjugates with radionuclides or toxic drugs).

Identifying genes encoding patient-specific, tumor-associated, surface-expressed molecules. The SEM approach described in the previous paragraph is combined with the combinatorial phage cDNA expression library approach to identify cDNAs that encode surface-expressed, patient-specific, tumor-associated antigens.

Targeted development of MAbs specific for the outer domain of membrane-localized growth factor receptors and cell-membrane transporter proteins. In this application, polyclonal antibodies produced against a nonexpressing parental cell line (driver) mask the same target cell genetically altered to express the surface membrane protein (tester), e.g., bombesin receptor, interferon receptors, and other proteins. SEM-derived MAbs would prove useful for both diagnostic and functional studies.

Development of primate monoclonal antibodies. In this approach, genetically altered CREF-Trans 6 cells are coated with high-titer CREF-Trans 6 polyclonal antibodies and injected directly into primates. Primate MAbs developed from immune-stimulated primate spleen cells should prove less immunogenic in humans than murine monoclonal antibodies (3).

CONCLUSION

With appropriate modifications, the SEM approach should also prove useful for producing MAbs and/or isolating relevant genes involved in tumor cell recognition by both nonspecific and specific immunologic effector cells, reacting with and encoding novel native and chimeric tumor-associated growth factor receptors, reacting with and encoding cell surface–localized tumor suppressor gene

products, mediating atypical multidrug resistance, and identifying mediators of autoimmune diseases.

Overall, SEM is a powerful new method of preparing monoclonal antibodies to tumor-associated antigens and other surface proteins of cells.

ACKNOWLEDGMENT

The author gratefully acknowledges the contributions of Drs. Neil I. Goldstein, Jorge Leon, Jian Lin, Sidney Pestka, Ruoqian Shen, and Zao-zhong Su to the studies briefly reviewed in this article. Research support was provided in part by Public Health Service grants CA35675 and CA43208 from the National Cancer Institute and by the Chernow Endowment.

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